

Suey J2

A method for treatment of Pythiosis in human

2 patients having the disease which comprises:

3 (a) providing a vaccine containing a mixture
4 of proteins of *Pythium insidiosum* in a sterile aqueous
5 solution, wherein the mixture of proteins is (1) of
6 mixed intracellular proteins removed from disrupted
7 cells of the *Pythium insidiosum* grown in a culture
8 medium and (2) of mixed extracellular proteins from the
9 culture medium for growing the *Pythium insidiosum* ;]
10 wherein the mixed intracellular proteins and the mixed
11 extracellular proteins in water have been dialyzed to
12 remove low molecular weight components less than 10,000
13 MW; and

14 (b) vaccinating the patient with the vaccine.

A method for the treatment of Pythiosis in a mammal having the disease which comprises:

(a) providing an injectable vaccine derived from growing cells of *Pythium insidiosum* in a culture medium which comprises in a sterile aqueous solution in admixture:

(1) mixed intracellular proteins removed from disrupted cells of the *Pythium insidiosum*; and

(2) mixed extracellular proteins removed from a supernatant from growing the cells of the *Pythium insidiosum*;

wherein the mixed intracellular proteins and the mixed extracellular proteins in water have been dialyzed to remove low molecular weight components less than 10,000 MW to product the vaccine; and

(b) vaccinating the mammal with the vaccine.

Sub 3 > 19- (Twice Amended)

1 The method of Claim 18 wherein the removed proteins
2 in the admixture have been provided by growing cells of
3 the *Pythium insidiosum* in the culture medium, then
4 killing the cells, then separating the killed cells from
5 the culture medium to produce a first supernatant
6 containing the mixed extracellular proteins and then
7 disrupting the killed cells in sterile water to provide
8 the mixed intracellular proteins in a second supernatant
9 and separating [removing] the mixed intracellular
10 proteins from the disrupted cells and removing the mixed
11 extracellular proteins from the first and second
12 supernatants [supernatant].

Sub 4 > 24- (Twice Amended)

1 The method of Claim 19 wherein the mixed
2 intracellular protein in the second supernatant are
3 separated from the disrupted cells [are removed from the
4 culture medium for the cells] by centrifugation and
5 removal of the disrupted cells [to provide the mixed
6 intracellular proteins in the second supernatant].